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(54) Title: TRANSGENIC RICE (ORYZA SATIVA) COMPRISING PE-7 EVENT AND METHOD OF DETECTION THEREOF

(57) Abstract: The present invention relates to an insect resistant transgenic rice plant, plant cell, seed and progeny thereof comprising a polynucleotide sequence encoding a CryIaC protein specific to PE-7 event. The invention also provides a process for detecting the presence of PE-7 event in transgenic rice plant. The invention further provides a kit for identifying the transgenic plants comprising the PE-7 event.



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## TRANSGENIC RICE (*Oryza sativa*) COMPRISING PE-7 EVENT AND METHOD OF DETECTION THEREOF

### FIELD OF INVENTION

The present invention relates to an insect resistant transgenic rice plant comprising PE-7  
5 event, wherein the event comprises the polynucleotide encoding for CryIAc protein.

### BACKGROUND OF INVENTION

Rice has been cultivated in the country for at least 5,000 years, and India is considered to be  
one of the centres of origin. The area under rice cultivation is estimated at 43.4 million ha,  
with a total production of 130,513,000 Mt (FAO data, 2005, <http://faostat.fao.org/>). Since  
10 1965, hundreds of high-yielding rice varieties have been released by the national rice  
research programs, adapted to different agro-climatic regions of the country.

In recent years, however, the production of rice in the Indian sub-continent has been  
affected by a steady increase in insect pest infestation, especially the yellow stem borer  
{*Scirpophaga incertulas*). The adult females lay eggs on leaves, and upon hatching, the  
15 young larvae bore into the culms (stems) of the rice plant and feed on the soft tissues within.  
This leads to the formation of 'dead hearts'. Infestations at later times in the crop cycle  
results in the formation of panicles with the absence of grain-filling, called chaffy ears. In  
Asia, there is a significant reduction in yield due to stem borers, of up to 10% overall, but  
local severe infestations can lead to crop losses of up to 70%.

20 Farmers use large quantities of chemical insecticides singly or in combination to control  
stem borers. This practice of indiscriminate use of insecticides leads to build up of pesticide  
residues on the grain, destruction of natural enemies, pest resurgence and environmental  
pollution.

To reduce pest-linked damage in rice crops as well as to protect the environment from  
25 adverse effects of pesticides, deploying the Lepidopteran specific *cryIAc* gene under the  
control of a suitable promoter for high level expression in rice would provide an effective  
built-in control for yellow stem borer. This would result in bringing down the cultivation  
costs of rice, as contribution of chemical pesticides to rice cultivation is significant.

A number of groups have carried out transformation of rice using different methods. The most successful of the methods are *Agrobacterium-mediated* methods for transformation (Azhakanandam K, McCabe MS, Power JB, Lowe KC, Cocking EC, and Davey MR., 2000, T-DNA Transfer, integration, expression and inheritance in rice: effects of plant genotype and *Agrobacterium* super-virulence *J. Plant Physiology* 157. 429-439).

The source organism for *cryIac* gene is *Bacillus thuringiensis* (Bt), which is a gram positive bacterium synthesizing insecticidal crystalline (Cry) inclusions during sporulation. The *cryIac* gene encodes the CryIac protein ( $\delta$ -endotoxins) of 130 kDa and is highly specific to Lepidopteran larvae. CryIac protein must be ingested by the insect to exhibit insecticidal activity. The protein in its crystalline form is insoluble in aqueous solution at neutral or acidic pH, however the pH of the larval insect gut is alkaline which favours solubilisation of the protein crystal. The solubilised protein is subsequently activated by the proteases in the insect gut. These proteases cleave the carboxy terminal domain from the rest of the protein as well as approximately 28 amino acids from the amino terminal end of the protein. The activated protein, which consists of approximately 600 amino acids, diffuses through the peritrophic membrane of the insect to the midgut epithelium. Here it binds to specific high affinity receptors on the surface of the midgut epithelium of target insects. Pores are formed in the membrane leading to leakage of intracellular content (eg. K<sup>+</sup>) into the gut lumen and water into the epithelial gut cells. The larval gut epithelial cells swell due to osmotic pressure and lyse. The gut becomes paralyzed as a consequence of changes in electrolytes and pH in the gut causing the larval insect to stop eating and die.

The expression of a foreign gene in plants is known to be influenced by the location of the transgene in the genome of the plant. Variations in transgene expression occur due to insertion into chromatin regions which may be more transcriptionally active (euchromatin) or less active (heterochromatin). Examples of these are methylated regions in which gene expression is suppressed or in the proximity of transcriptional regulation elements like enhancers and suppressor, which increase or decrease gene expression respectively. Therefore it is necessary to screen a large number of independent transformation event for the expression of the transgene and to identify the event showing desired expression of the heterologous inserted gene.

## SUMMARY OF INVENTION

The present invention relates to an insect resistant transgenic rice plant comprising PE-7 event comprising the polynucleotide encoding for CryI $\text{Ac}$  protein. The transgenic rice plant comprises *cryI $\text{Ac}$*  gene under the control of CaMV 35S promoter at a specific locus in the rice genome. Further, the invention relates to a method for detection of the PE-7 event in transgenic rice plant. The present invention relates to an isolated polynucleotide sequences useful for detection of the PE-7 event. The invention further provides a kit for identifying the transgenic plants comprising the PE-7 event.

One aspect of the present invention is to provide an isolated polynucleotide useful for detection of presence of PE-7 event in a sample comprising rice DNA, wherein the nucleotide sequence of the polynucleotide is selected from the group consisting of SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15 and a complement thereof.

Another aspect of the present invention is to provide a set of synthetic oligonucleotides comprising first and second oligonucleotide useful for detection of presence of PE-7 event in a sample comprising rice DNA, wherein the first oligonucleotide comprises at least 15 nucleotides from position 31 to position 850 of the nucleotide sequence as set forth in SEQ ID NO: 7 and the second oligonucleotide comprises at least 15 nucleotides from position 900 to position 1759 of the nucleotide sequence as set forth in SEQ ID NO: 7.

Yet another aspect of the present invention is to provide a set of synthetic oligonucleotide useful for detection of presence of PE-7 event in a sample comprising rice DNA, wherein the nucleotide sequence of the oligonucleotide is as set forth in SEQ ID NO: 8 and SEQ ID NO: 4.

Still yet another aspect of the present invention is to provide a set of synthetic oligonucleotide useful for detection of presence of PE-7 event in a sample comprising rice DNA, wherein the nucleotide sequence of the oligonucleotide is as set forth in SEQ ID NO: 8 and SEQ ID NO: 9.

Further aspect of the present invention is to provide a set of synthetic oligonucleotide useful for detection of presence of PE-7 event in a sample comprising rice DNA, wherein the nucleotide sequence of the oligonucleotide is as set forth in SEQ ID NO: 8 and SEQ ID NO: 10.

Another aspect of the present invention is to provide a process of detecting the presence of PE-7 event in a sample comprising rice DNA, the method comprising:

- contacting said sample with a set of synthetic oligonucleotides comprising first and second oligonucleotide useful for detection of presence of PE-7 event in a sample comprising rice DNA, wherein the first oligonucleotide comprises at least 15 nucleotides from position 31 to position 850 of the nucleotide sequence as set forth in SEQ ID NO: 7 and the second oligonucleotide comprises at least 15 nucleotides from position 900 to position 1759 of the nucleotide sequence as set forth in SEQ ID NO: 7.
- performing amplification reaction; and
- 10• detecting the presence or absence of an amplicon.

wherein the presence of an amplicon confirms the presence of PE-7 event in the sample.

Yet another aspect of the present invention is to provide a process of detecting the presence of PE-7 event in a sample comprising rice DNA, the process comprising:

- contacting the sample with a polynucleotide probe having nucleotide sequence as set forth in SEQ ID NO: 12, SEQ ID NO: 13 or SEQ ID NO: 14
- subjecting the sample and said probe to stringent hybridization conditions; and
- detecting hybridization of said probe to the DNA.

wherein the hybridization signals confirms the presence of PE-7 event in the sample.

- 20 Further aspect of the present invention is to provide a kit for detection of presence of PE-7 event in a sample, wherein said kit comprising a set of synthetic oligonucleotides comprising first and second oligonucleotide, wherein the nucleotide sequence of the first oligonucleotide is as set forth in SEQ ID NO: 8 and the nucleotide sequence of the second oligonucleotide is selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 9 and SEQ ID NO: 10.

- 25 Another aspect of the present invention is to provides a kit for detection of presence of PE-7 event in a sample, wherein the kit comprising a polynucleotide probe having nucleotide sequence as set forth in SEQ ID NO: 12, SEQ ID NO: 13 or SEQ ID NO: 14

**BRIEF DESCRIPTION OF ACCOMPANYING DRAWINGS**

Figure 1 shows a map of the construct pMH0102

Figure 2 shows gel image of the PE-7 event using the event specific primers

Figure 3 Southern blot analysis of transgenic rice plant comprising PE-7 event

5           A)     *Hmd*III digested genomic DNA of rice plant comprising PE-7 event

              B)     *Dra* I digested genomic DNA of rice plant comprising PE-7 event

**DETAILED DESCRIPTION OF THE INVENTION**

The present invention relates to an insect resistant transgenic rice plant comprising a PE-7 event, wherein the event comprises *cryIAC* gene under the control of CaMV e35S promoter  
10     at a specific locus in the rice genome. Further, the invention discloses a process for detection of PE-7 event in transgenic rice plants, plant cells, tissues or seeds thereof. The invention further provides a kit for identifying the transgenic plants comprising the PE-7 event.

The term "amplicon" or "amplified DNA" "amplified fragment" refers to the product of  
15     nucleic acid amplification of a target nucleic acid sequence that is a part of nucleic acid template.

The term "Heterologous Gene/DNA" refers to DNA sequence of foreign origin inserted in the plant genome.

The term "event" refers to the original transformant and any progeny produced by a sexual  
20     outcross between the original transformant or its descendants bearing the heterologous gene, and another rice variety.

The term "probe" refers to a DNA sequence identical to the gene of foreign origin inserted in the plant genome.

The present invention relates to transformation of rice plant with *cryIAC* gene for conferring  
25     insect resistance. The invention pertains to transforming rice plants with plant expression vectors pMH0102 and pCAMBIA1201 by *Agrobacterium-mediated* transformation method.

In one embodiment of the present invention provides more than 55 independent transformation events comprising the *cryIAc* gene. All the independent events were screened and characterized for the expression of the CryIAc protein. Based on the level of expression of the CryIAc protein and insect bioassays, three events were selected for further characterization. Of these three events one specific event showing optimum expression of CryIAc protein was designated as PE-7 event that was further characterized.

The present invention also relates to a process of identification of transformation events.

The specific location of the insertion of the heterologous gene was analyzed by molecular methods. This involves cloning of the genomic region flanking the left border of the T-DNA into suitable vectors.

The present invention also relates to analysis of the flanking region by sequencing. The present invention further relates to primers those were designed from this region of the DNA sequence for amplification of the genomic DNA of rice PE-7 event.

In accordance with the present invention, one embodiment of the present invention is to provide a DNA construct for rice transformation, wherein the construct comprises the polynucleotide sequence coding for CryIAc protein under the control of CaMV e35S promoter sequence.

Another embodiment of the present invention is to provide a transformation vector comprising the DNA construct comprising the polynucleotide sequence coding for CryIAc protein under the control of CaMV e35S promoter sequence.

Yet another embodiment of the present invention is to provide a host cell comprising the DNA construct comprising the polynucleotide sequence coding for CryIAc protein under the control of CaMV e35S promoter sequence, wherein the host cell is selected for a group consisting of *E. coli*, *Agrobacterium* and yeast.

Still yet another embodiment of the present invention is to provide a method for transformation of rice plant, cells and tissues using the transformation vector disclosed in the present invention.

The present invention provides a method for transforming plant, plant cells and tissues of Rice (*Oryza sativa*) using *Agrobacterium-mediated* transformation method using the

transformation vector disclosed in the present invention for conferring resistance to insect pests.

Further embodiment of the present invention provides the transgenic plant comprising the polynucleotide sequence coding for Cry IAc protein.

- 5 Preferred embodiment of the present invention provides a transgenic rice plant comprising the PE-7 event comprising the polynucleotide coding for Cry IAc protein.

One of the preferred embodiments of the present invention provides the primer sequences for identification of the PE-7 event in transgenic rice plant.

- 10 Another preferred embodiment of the present invention is to provide a kit for detection of PE-7 event in transgenic rice plant.

The present invention further provides a diagnostic tool to distinguish the rice PE-7 event from other rice transformation events and non-transgenic rice plants.

- 15 One embodiment of the present invention provides the explants for transformation are selected from a group consisting of embryogenic calli derived from scutelar part of mature seed, embryo, immature embryo, leaf lamina, shoot tip, anther and root or any other suitable explant.

- 20 Another embodiment of the present invention is to provide a process of identification of the flanking sequence around the transgenic insertion site for PE-7 event by nucleic acid amplification. Nucleic acid amplification can be accomplished by any of the various nucleic acid amplification methods known in the art, including the polymerase chain reaction (PCR).

Transgenic insertion and neighbouring flanking rice DNA were purified by agarose gel electrophoresis and cloned. The cloned fragment was sequenced by methods known in the art.

- 25 Another embodiment of the present invention is to provide diagnostic methods for identification of PE-7 event in transgenic rice plants.

Another embodiment of the present invention is to provide a method of introduction of PE-7 event in other background or cultivars.

Another embodiment of the present invention is to provide a method of production of hybrids using the transgenic rice having PE-7 event.

Yet another embodiment of the present invention provides a novel DNA molecule having polynucleotide sequence as set forth in the SEQ ID NO: 7 and the complement thereof.

- 5 The present invention further provides a method of producing transgenic rice plant resistant to insect pests comprising transforming a rice cell with the DNA construct pMH0102. The fertile rice plant obtained from the rice cell can be self pollinated or crossed with compatible rice varieties to produce insect resistant rice plant.

- 10 The present invention provides an efficient method for transforming plant, plant cells and tissues of Rice (*Oryza sativa*) using *Agrobacterium-mediated* transformation method for conferring resistance to insect pests.

Transgenic insertion and neighbouring flanking rice DNA were purified by agarose gel electrophoresis and the purified fragment was cloned in a vector. The cloned fragment was sequenced by methods known in the art.

- 15 Another embodiment of the present invention is to provide a method of introduction of PE-7 event in other background or cultivars.

Another embodiment of the present invention is to provide a method of production of hybrids using the transgenic rice having PE-7 event.

- 20 Insecticidal *cryIAc* gene from *Bacillus thuringiensis* has been transferred into rice line MPH-I developed by MAHYCO. The present invention provides an efficient method for transforming plant, plant cells and tissues of rice (*Oryza sativa*) plant using *Agrobacterium-mediated* transformation method for conferring resistance to insect pests.

- 25 The explants for *Agrobacterium* mediated transformation of rice plant were selected from a group consisting of embryogenic calli derived from scutellar part of mature seed, embryo, immature embryo, leaf lamina, shoot tip, anther and root or any other part of plant.

The vector pMH0102 (Figure 1) comprising *cryIAc* gene under the control of a CaMV e35S promoter and VelO518 polyA terminator; and the vector pCAMBIA1201 containing *hpt* gene under the control of a CaMV 35S promoter as a plant selectable marker gene and a *GUS* gene under the control of a CaMV 35S promoter were introduced into *Agrobacterium*

*tumefaciens*. The recombinant *A. tumefaciens* was inoculated into a suitable medium for growth of *Agrobacterium*. *Agrobacterium* cells were inoculated into 25 ml of sterile LB medium (pH 7) in a flask. LB medium contains 1% Tryptone; 0.5% Yeast extract and 1% NaCl with pH 7.0. Suitable antibiotics were added to this medium before inoculating  
5 bacteria for the selective growth of *Agrobacterium* with the plasmids pMH0102 and pCAMBIA1201. The bacteria were inoculated in LB medium and grown with shaking until the culture reached an optical density (600nm) in the range of 1.5 to 2, preferably 1.8.

Explants were inoculated in recombinant *Agrobacterium* suspension (preferably 15 minutes), blotted dry on sterile filter paper and later transferred to petri plates containing  
10 suitable growth medium for co-cultivation. After the co-cultivation (about 3 days of co-cultivation), these explants were transferred on post-culture medium, LS-Cef containing Cefotaxime. After about 10 days of post-culture, the explants were transferred on to selection medium LS-H50 with Hygromycin and Cefatoxime for a period of about 2 weeks. Putative transformants were maintained on fresh selection medium LS-H50 again for about  
15 two weeks and transferred on to regeneration I medium, RI- MSB2.5 with Hygromycin and Cefotaxime for a period about 10 days. These transformants were regenerated and elongated on regeneration II medium, RII- MSB2.5. Elongated transformants were transferred to rooting medium and the rooted plants were hardened and established in the greenhouse.

20 In accordance with the present invention, one embodiment provides an isolated polynucleotide useful for detection of presence of PE-7 event in a sample comprising rice DNA, wherein the nucleotide sequence of the polynucleotide is selected from the group consisting of SEQ ID NO: 12, SEQ BD NO: 13, SEQ ID NO: 14, SEQ ID NO: 15 and a complement thereof.

25 Another embodiment of the present invention provides a set of synthetic oligonucleotides comprising first and second oligonucleotide useful for detection of presence of PE-7 event in a sample comprising rice DNA, wherein the first oligonucleotide comprises at least 15 nucleotides from position 31 to position 850 of the nucleotide sequence as set forth in SEQ ID NO: 7 and the second oligonucleotide comprises at least 15 nucleotides from position  
30 900 to position 1759 of the nucleotide sequence as set forth in SEQ ID NO: 7.

Yet embodiment of the present invention provides the first oligonucleotide is as set forth in SEQ ID NO: 8.

Still yet another embodiment of the present invention provides the second oligonucleotide is selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 9 and SEQ ID NO: 10.

Further embodiment of the present invention provides a set of synthetic oligonucleotide useful for detection of presence of PE-7 event in a sample comprising rice DNA, wherein  
5 the nucleotide sequence of the oligonucleotide is as set forth in SEQ ID NO: 8 and SEQ ID NO: 4.

Another embodiment of the present invention provides a set of synthetic oligonucleotide useful for detection of presence of PE-7 event in a sample comprising rice DNA, wherein the nucleotide sequence of the oligonucleotide is as set forth in SEQ ID NO: 8 and SEQ ID  
10 NO: 9.

Another embodiment of the present invention provides a set of synthetic oligonucleotide useful for detection of presence of PE-7 event in a sample comprising rice DNA, wherein the nucleotide sequence of the oligonucleotide is as set forth in SEQ ID NO: 8 and SEQ ID NO: 10.

15 One embodiment of the present invention provides a process of detecting the presence of PE-7 event in a sample comprising rice DNA, the method comprising:

- contacting the sample with a set of synthetic oligonucleotides comprising first and second oligonucleotide useful for detection of presence of PE-7 event in a sample comprising rice DNA, wherein the first oligonucleotide  
20 comprises at least 15 nucleotides from position 31 to position 850 of the nucleotide sequence as set forth in SEQ ID NO: 7 and the second oligonucleotide comprises at least 15 nucleotides from position 900 to position 1759 of the nucleotide sequence as set forth in SEQ ID NO: 7.
- performing amplification reaction; and
- 25 • detecting the presence or absence of an amplicon, wherein the presence of an amplicon confirms the presence of PE-7 event in said sample.

Another embodiment of the present invention provides the process of detecting the presence of PE-7 event in a sample comprising rice DNA using first and second oligonucleotides, wherein the first oligonucleotide is as set forth in SEQ ID NO: 8.

Another embodiment of the present invention provides the process of detecting the presence of PE-7 event in a sample comprising rice DNA using first and second oligonucleotides, wherein the second oligonucleotide is selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 9 and SEQ ID NO: 10.

5 The present invention further provides a process of detecting the presence of PE-7 event in a sample comprising rice DNA, the process comprising:

- contacting the sample with a polynucleotide probe having nucleotide sequence as set forth in SEQ ID NO: 12, SEQ ID NO: 13 or SEQ ID NO: 14;
- 
- 10 • subjecting the sample and the probe to stringent hybridization conditions; and
- detecting hybridization of the probe to said DNA, wherein the hybridization signals confirms the presence of PE-7 event in the sample.

The present invention further provides a kit for detection of presence of PE-7 event in a sample, wherein the kit comprising a set of synthetic oligonucleotides comprising first and  
15 second oligonucleotide, wherein the nucleotide sequence of the first oligonucleotide is as set forth in SEQ ID NO: 8 and the nucleotide sequence of the second oligonucleotide is selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 9 and SEQ ID NO: 10.

The present invention also provides a kit for detection of presence of PE-7 event in a sample, wherein the kit comprising a polynucleotide probe having nucleotide sequence as  
20 set forth in SEQ ID NO: 12, SEQ ID NO: 13 or SEQ ID NO: 14

In another embodiment, the present invention provides a plant cell or a progeny thereof, wherein the plant cell, or the progeny thereof comprising PE -7 event.

In the present invention, the *cryIAc* gene from *Bacillus thuringiensis* has been transferred into rice line MPH-I developed by MAHYCO. A detailed procedure of co-transformation of  
25 rice plant with the pMH0102 and pCAMBIA1201 constructs is provided in Example 1.

More than 55 independent transformation events were screened to identify rice plant designated as PE-7 event. All events underwent transgene segregation analysis and protein expression evaluation to determine the optimum event for commercialization. Details are

provided in Example 2. Molecular characterization of the rice plant comprising PE-7 event was carried out. Details are provided in the Example 3.

5 Southern blot analysis of selected individual transformation events was carried out to confirm the number of loci (insert number) at which the transgene integrated in the rice genome. Detailed procedure is provided in Example 4.

Further the present invention also provides diagnostic methods for identification of rice plants comprising the PE-7 event was carried out, the methods are described in detail in Example 5.

10 Detailed description of the zygosity assay developed for transgenic rice plant comprising PE-7 event is provided in Example 6.

The transgenic rice plant comprising PE-7 event was chosen on the basis of a number of criteria. Segregation analysis of the transgenic rice plant comprising the PE-7 event over three generations indicated that there is a single locus of insertion of the *cryIAC* gene in this line. This was confirmed by DNA blot analysis. Protein quantification of number of rice  
15 single insertion events was carried out using quantitative ELISA method. Surprisingly it was found that the transgenic rice plant comprising PE-7 event was the highest Cry IAC protein expressing lines and expression of the *cryIAC* gene was stable in a number of different genetic backgrounds, over multiple generations. Phenotypic analysis of the transgenic rice plant comprising the PE-7 event showed that it was morphologically  
20 indistinguishable from the non-transformed parent line from which it was derived and therefore most suitable for further backcross breeding.

It should also be understood that the foregoing relates to preferred embodiments of the present invention and that numerous changes may be made therein without departing from the scope of the invention. The invention is further illustrated by the following examples,  
25 which are not to be construed in any way as imposing limitations upon the scope thereof. On the contrary, it is to be clearly understood that resort may be had to various other embodiments, modifications, and equivalents thereof, which, after reading the description herein, may suggest themselves to those skilled in the art without departing from the spirit of the present invention and/or the scope of the appended claims.

30

## EXAMPLES

It should be understood that the following examples described herein are for illustrative purposes only and that various modifications or changes in light will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and the scope of the appended claims.

**Example 1****Transformation of Rice**Sterilization and inoculation of seeds

Rice seeds from line MPH-I were surface sterilised in a 250 ml conical flask with 1.2 % NaOCl for 20 min. with vigorous shaking (30 ml NaOCl for 500 seeds). After 20 min the solution was decanted and the seeds washed 5 times with sterile distilled water. The seeds were blotted dry on sterile filter paper for 1 hr and inoculated on LS medium (Table 1) in plastic plates at 10 seeds/plate. The seeds were maintained at 28°C in dark for about 14 days to produce calli.

15 *Asrobacterium* cultures

A day before co-cultivation was to be done, a culture of the *Agrobacterium* strain harbouring the transformation vectors pMH0102 and pCAMBIA1201 comprising the polynucleotide sequence coding for CryIAc protein was grown overnight in 25 ml liquid LB medium (Table 1) at 28°C with shaking at 175 rpm with antibiotics. This overnight culture was started with a loopful of bacterial cells taken from a freshly-streaked solid medium plate containing the same antibiotics.

Calli isolation and pre-culture

About three days before co-cultivation, the calli of about 14 days old were isolated from the seeds and cut into pieces of size 3-4 mm<sup>2</sup> and pre-cultured on LS (Table 1) solid media for co-cultivation. The cultures were maintained at 28°C in dark for about 3 days.

Co-cultivation

Prior to co-cultivation optical density (O.D.) of the overnight grown *Agrobacterium* culture was measured at 600 nm. The optical density of the overnight grown bacterial culture was adjusted to 1.00 using liquid LS medium. The pre-cultured calli were incubated in the recombinant *Agrobacterium* culture in a petridish or a glass beaker (50 ml beaker) for 15

min with slow intermittent stirring. The calli were blotted on a sterile filter paper to remove excess bacteria and cultured on co-cultivation medium LS-As (Table 1) for about three days for co-cultivation (20 explants per plate). The cultures were incubated in dark at 28°C for about three days.

- 5 Positive and negative controls were also maintained in each experiment. Positive controls were explants cultured on medium without antibiotics to check the tissue culture regeneration, whereas negative controls were explants maintained on antibiotic-containing media to make sure the antibiotic is checking the growth.

After about 3 days of co-cultivation, the calli were transferred on to postculture medium,  
10 LS-Cef (Table 1). The cultures were incubated in dark at 28°C for a period of about 10 days.

#### Selection (LS-H50 medium) of the putative transgenic rice calli

- After about 10 days of postculture, the calli were transferred on to the selection medium LS-H50 (Table 1) with Hygromycin 50 mg/1 and Cefatoxime 250 mg/1 for a period of about 2  
15 weeks. Putative transformed calli were grown and produced protuberances and non-transformed calli became dark brown, black. Putative transformed calli were maintained on fresh selection medium LS-H50 again for a period of about two weeks. Cultures were incubated in dark at 28°C.

#### Regeneration I (RI- MSB2.5 medium)

- 20 Golden white calli were transferred on to the regeneration I medium, RI- MSB2.5 (Table 1), with Hygromycin 40 mg/1 and Cefotaxime 250 mg/1 for a period of about 10 days. Cultures were incubated in dark at 28°C. At this stage, putative transgenic calli started producing bright protuberances that turned into small shoot buds while non-transgenic calli turned completely black and did not produce protuberances or shoot buds.

- 25 Regeneration II (RII- MSB2.5 medium)

After about 10 days on regeneration I medium, the calli were transferred on to the regeneration II medium, RII- MSB2.5 (Table 1), with Hygromycin 30 mg/1 and Cefotaxime 250 mg/1, for shoot regeneration. Cultures were incubated at 28°C with a photoperiod regime of 16 hrs light + 8 hrs darkness for a period of about 10 days. At this stage small

green shoot buds were visible from putative transgenic calli. These shoot buds were grown on this medium.

#### Elongation (RII- MSB2.5 medium)

Small shoots were transferred on to the fresh medium RII- MSB2.5 with Hygromycin 30 mg/lit and Cefotaxime 250 mg/lit for a period of about 2 weeks, for shoot multiplication and elongation. Cultures were incubated at 28°C with a photoperiod regime of 16 hrs light + 8 hrs darkness.

#### Rooting (MSN 1.5 medium)

The individual shoots were transferred on to the rooting medium MSN1. 5 (Table 1) with Hygromycin 20 mg/1 and Cefotaxime 250 mg/1 for a period of about 2 weeks. The shoots were subcultured about every 2 weeks till healthy rooting.

#### Hardening

The rooted plants were washed with sterile distilled water thoroughly to remove the gelling agent (phytagel). Plants were transferred to cups containing mixture of promix (60%) and soil (40%). The plants were covered with polythene bags for a period of about 7 days. After about 7 days, the polythene bags were cut from the corners to allow the hardening process to begin, which was completed in about 2 weeks.

### **Example 2**

#### **Identification of rice plant PE-7 event**

A large number (>55) of independent transformation events were generated in order to maximize the chance of a high-transgene-expressing, genetically stable event for production of commercial transgenic rice lines. All rice plants coming out of the transformation experiments were analyzed for presence of the *cryIAc* gene by PCR and positive plants were subjected to ELISA for determining the expressivity of the transgene. The initial transformants ( $T_0$ ) were advanced to the next generation by selfing and the Ti progeny plants were checked by PCR to determine the segregation of the transgene. The expected Ti segregation ratio for the transgene in a line with a single *cryIAc* gene insertion is 3:1 based on Mendelian genetics. Further, as *cryIAc* gene acts as a dominant gene when introduced as a transgene, the expression of the gene was monitored by ELISA in the Ti generation. Again, in a single insertion event, the expected ratio of CryIAc expressing plants to non-

expressing plants is 3:1. Insect bioassays were carried out on tissue from selected lines in order to determine which lines would have better efficacy against the yellow stem borer pest. Based on the above criteria, transformed lines were selected which displayed segregation characteristics of single locus insertion events and showed effective tolerance to yellow stem borer. Conversely, those lines that were found to have abnormal segregation ratios and/or low efficacy against the pest were not taken further. The lines selected for advancement were grown in the greenhouse and CryI<sub>Ac</sub> protein was estimated through the life of the crop by quantitative ELISA, which enables determination of the highest protein expressing lines. The tissues analyzed were leaf, culm, panicle and root. The line showing the optimum expression of CryI<sub>Ac</sub> protein was selected out of more than 55 independent transgenic rice plants and was designated as PE-7 event.

After a careful analysis of the above parameters, event PE-7 was found to be one of the best available events, in terms of CryI<sub>Ac</sub> expression, efficacy against the pest and genetic stability over three plant generations. The PE-7 event was used for further breeding for developing yellow stem borer-tolerant rice.

### Example 3

#### Molecular characterization of the transgenic rice plant comprising the PE-7 event

The transgenic rice plant comprising the PE-7 event was analyzed to identify rice genomic DNA sequences flanking the *cryI<sub>Ac</sub>* gene expression cassette using the method described by Cottage et al. (Cottage A, Yang, A, Maunders H, de Lacy RC, and Ramsay NA. 2001, Identification of DNA sequences flanking T-DNA insertions by PCR-walking. *Plant Molecular Biology Reporter* 19:321-327).

Plant genomic DNA was extracted from fresh young leaves of transgenic plant comprising PE-7 event (Dellaporta S, Wood J, and Hicks J B, 1983 A plant DNA miniprep: version II. *Plant Molecular Biology Reporter* 1:19-21). Genomic DNA (2µg) was digested with *Ssp*I enzyme in 20 µl of reaction volume using standard buffers. The digestion reaction was incubated at 37°C overnight. The digestion product was then incubated at 65°C for enzyme inactivation and was precipitated with 3M sodium acetate and ethanol. DNA was air dried and dissolved in 12 µl sterile distilled water. Digested DNA was ligated to the annealed adapter in ligase buffer supplied by the manufacturer. The sequences of the adapters are as below

ADAP 1: 5'-CTAATACGACTCACTATAGGGCTCGAGCGGCCCGCCGGGCAGGTS'-SEQ ID. NO: 1

ADAP 2: 5'-P-ACC TGC CC-H<sub>2</sub>N -3'

SEQ ID NO: 2

Both the adapters were first annealed to each other and ligated to the digested genomic DNA of PE-7 event.

- 5 The ligation mixture was incubated at 15-16°C overnight for ligation of digested genomic DNA to the annealed adapters. The ligation mixture was diluted to 100 µl for obtaining adapter library, and first round amplification was carried out using the following primer combination: Forward primer complementary to the adapter DNA sequence SEQ ID NO: 3 and reverse primer complementary to the inserted heterologous DNA SEQ ID NO: 4.

10 AP: 5'-GGATCCTAATACGACTCACTATAGGGCS'- SEQ ID NO: 3

MHIP-5: 5'-GACCTGCAGCCAAGCTTCG -3'- SEQ ID NO: 4

#### Restriction digestion

Genomic DNA	12.0 µl (2 µg)
10X Reaction buffer	2.0 µl (final concentration 1x)
<i>Ssp</i> I enzyme	1.0 µl (10units/ µl)
Sterile water	Make up the volume to 20.0 µl.

#### 15 Ligation:

Digested Genomic DNA (heat inactivated)	12.0 µl (2 µg)
Annealed adapters	2.0 µl (100 ng/µl)
10X Reaction buffer	3.0 µl (final concentration 1x)
T4-ligase enzyme	1.0 µl (5units/ µl)
Sterile water	Make up the volume to 30.0 µl

#### First PCR:

Reagents	Volume
Nuclease-free water	Make up to 25 µl
10X reaction buffer (with MgCl <sub>2</sub> )	2.5 µl
10mM dNTPs	0.5 µl
Primer AP (100 ng/µl)	1.0 µl
Primer MHIP-5 (100ng/µl)	1.0 µl
Taq DNA polymerase (5 units/µl)	0.5 µl
DNA template	3.0 µl

#### Thermal Cycler program:

Temperature	Time	Cycles
95°C	5 minutes	1

94°C	30 seconds	
58°C	30 seconds	<b>40</b>
68°C	4 minutes	
68°C	10 minutes	<b>1</b>
4°C	Hold	

The second round of PCR was necessary to obtain the specific flanking region adjacent to the inserted heterologous gene. PCR was carried out with forward primer (SEQ ID NO: 5) and a reverse primer (SEQ ID NO: 6). Details are given below:

NAP: 5'- TATAGGGCTCGAGCGGCS' SEQ ID NO: 5

5 MHIP-6: 5'CAAGCTTCGAATTAATTCAGTACS' SEQ ID NO: 6

Second PCR:

Reagents	Volume
Nuclease-free water	Make up to 25 µl
10X reaction buffer (with MgCl <sub>2</sub> )	2.5 µl
10mM dNTPs	0.5 µl
Primer NAP (100ng/µl)	1.0 µl
Primer MHIP-6 (100ng/µl)	1.0 µl
Taq DNA polymerase (5 units/µl)	0.5 µl
DNA template	2.0 µl

Thermal Cycler program:

Temperature	Time	Cycles
95°C	5 minutes	1
94°C	30 seconds	
58°C	30 seconds	40
68°C	4 minutes	
68°C	10 minutes	1
4°C	Hold	

A small amount of PCR product was analyzed on a 1% agarose gel, and the amplified fragment was eluted from the gel by using the method known in the art. A DNA fragment (amplicon) of 939 bp (SEQ ID NO: 16) was amplified from the left border region of the T-DNA after two rounds of PCR (using primers NAP SEQ ID NO:5 and MHIP-6 SEQ ID NO: 6). The amplified fragment (amplicon) was cloned into pGEM-T Easy vector to obtain a recombinant vector. This recombinant vector was transformed in the strain of *E. coli* by using method known in the art. The strains can be DH5 $\alpha$ , Top 10 etc. The clone comprising this recombinant vector selected for analyzing the sequence was designated as PE-7-*Sspl*-10. Plasmid DNA from the clone PE-7-S.spI-IO was isolated using standard methods known in the art. The cloned fragment (amplicon) was sequenced. The sequence in SEQ ID NO: 7 contains the adapter sequence, PE-7 T-DNA flanking rice genomic DNA sequence, a part of

left border, e 35 S promoter and a part of *cryIAC* gene coding sequence.

#### Example 4

##### Southern Blot analysis

##### PE-7 genomic DNA digestion with *Hind III*

- 5 A unique *Hind III* site is present in pMH0102 plasmid (Figure 1). For a single copy and single insertion of pMH0102 T-DNA in rice PE-7 event, *Hind III* digestion was expected to yield a single fragment. Upon digestion of the PE-7 rice genomic DNA with *Hind III* and hybridizing with PCR amplified *cryIAC* fragment as a probe, a single fragment of approximately 9.2kb was generated (Figure 3A). This indicates a single copy and single
- 10 insertion of the pMH0102 T-DNA in rice event PE-7. The hybridized fragment consisted of promoter e35S, *cryIAC* gene, terminator, right border and rice plant genomic DNA.

##### PE-7 genomic DNA digestion with *Dra I*

- A unique *Dra I* site is present in the T-DNA region of plasmid pMH0102 (Figure1). For a single copy and single insertion of pMH0102 T-DNA in rice event PE-7, *Dra I* digestion
- 15 was expected to yield a single fragment. Upon digestion of genomic DNA of the PE-7 rice event with *Dra I* and hybridizing with PCR amplified *cryIAC* fragment as a probe, a single fragment of approximately 4.8kb was generated (Figure 3 B). This indicates a single copy and single insertion of pMH0102 T-DNA in rice event PE-7. The hybridized fragment consists of promoter e35S, *cryIAC* gene, terminator, part of right border, left border and rice
- 20 plant genomic DNA.

##### Inheritance and stability of the *cryIAC* gene in subsequent generations

- The inheritance and stability of expression of the *cryIAC* gene was analysed for several generations using ELISA technique. Young leaf samples were ground with PBST buffer. The expression of the CryIAC protein was detected by double antibody sandwich (DAS)
- 25 ELISA technique. Segregation data over two generations for the PE-7 event showed that the *cryIAC* gene was stably integrated in the PE-7 event (Table 1).

**Table 1: Segregation data in different generations:**

S. No.	Generation tested	Elisa positive	Elisa Negative
1	T <sub>1</sub>	21	4
2	T <sub>2</sub>	15	1

**Example 5****Detection of PE-7 event in transgenic rice plant**

To detect the presence or absence of the rice PE-7 event, a molecular diagnostic method was developed. The sequence analysis of the fragment shown as SEQ ID NO: 7 was carried  
 5 out and the primers were designed to amplify the transgenic insertion locus for use as a diagnostic tool. The two primers designed were forward primer MHTPH-7 (SEQ ID NO: 8) and the second primer is MHIP-5 (SEQ ID NO: 4) to amplify the transgenic insertion locus from PE-7 genomic DNA.

MHTPH-7: 5' CAC ATG CAT GCT CTG AAA CC -3' SEQ ID NO: 8

10 These primer pairs include, but are not limited to, SEQ ID NO: 8 and SEQ ID NO: 4. For the amplification of the 5' region, any primer pair derived from SEQ ID NO: 7 that when used in DNA amplification reaction produces a DNA amplicon diagnostic for PE-7 event is an aspect of the present invention.

However, any modification of these methods that use DNA molecules or complements  
 15 thereof to produce an amplicon DNA molecule diagnostic for PE-7 is within the ordinary skill of the art. For example if SEQ ID: 8 primer if used in combination with primer 1 (SEQ ID NO: 9) will produce an amplicon of 1206 base pair, or in combination with primer 2 (SEQ ID NO: 10) will amplify 1255 base pair from PE-7 event. The sequences of primer 1 and 2 are as below.

20 Primer 1: 5' GCATTCGTTGATGTTTGGGTGS' SEQ ID NO: 9

Primer 2: 5' CTCCACCAAGTACTTCAACTCS' SEQ ID NO: 10

For the analysis it is important to have positive and negative controls. The PCR method was designed in order to distinguish the PE-7 event from the other rice transgenic events and non-transgenic lines. Genomic DNA from rice PE-7 event was isolated from leaves using  
 25 the method described by Dellaporta *et al.* (1983). Genomic DNA was also isolated from other rice transgenic events and non-transgenic rice lines as controls for the PCR detection method. A control reaction having no DNA in the reaction mixture was also included.

The genomic DNA from different plants was subjected to amplification using two primers namely SEQ ID NO: 8 and SEQ ID NO: 4, the details are as follows:

Reagents	Amount to be added
Nuclease-free water	Make up to 25 $\mu$ l
10X reaction buffer (with $MgCl_2$ )	<b>2.5</b> $\mu$ l
10mM dNTPs	<b>0.5</b> $\mu$ l
Primer MHTPH-7 (100 ng/ $\mu$ l)	1.0 $\mu$ l
Primer MHIP-5 (100 ng/ $\mu$ l)	1.0 $\mu$ l
Taq DNA polymerase (5 units/ $\mu$ l)	<b>0.5</b> $\mu$ l
DNA template	<b>2.0</b> $\mu$ l

Thermal Cycler program:

Temperature	Time	Cycles
95°C	<b>5</b> minutes	1
94°C	<b>30</b> seconds	<b>35</b>
60°C	<b>30</b> seconds	
72°C	1minute	
72°C	<b>5</b> minutes	1
4°C	Hold	---

The amplified product was analyzed on agarose gel electrophoresis. The results obtained are shown in Figure 2. Lane 1, 2, 3 and 5 contains rice genomic DNA from other transgenic rice lines that do not contain the PE-7 event. The sample in lane 4 contains the genomic DNA from the rice PE-7 event. Lane 6 represents a non-transgenic rice control, and lane 7 is a control and has no DNA and lane 8 represents of molecular weight marker. From the figure it is evident that the 566 bp fragment is amplified from the rice PE-7 event but not from other transgenic events and non-transgenic rice plants.

- Lane 1, 2, 3 and 5. DNA from transgenic rice plants that do not contain the PE-7 event
- 10 Lane 4. PE-7
- Lane 6. DNA template from a non-transgenic rice plant
- Lane 7. No DNA (water) - negative control
- Lane 8. Molecular weight marker

### Example 6

- 15 Zygosity assay for rice PE-7 event

The PE-7 flanking genomic DNA sequence described in Example 3 was used to obtain sequence information adjacent to the T-DNA. A primer was designed having nucleotide sequence as shown in SEQ ID NO: 11.

MHTPH-8: 5'TTCATCCGTTCTGTGGACG'3      SEQ ED NO: 11

This primer when used in combination with the primer having nucleotide sequence as set forth in SEQ ID NO: 8 and SEQ ID NO: 4 results in the amplification of the following fragments:

1) 566 base pairs (transgene specific band from SEQ ID NO: 8 and SEQ ID NO: 4)

52) 826 base pairs (non-transgenic allele band from SEQ ID NO: 8 and SEQ ID NO: 11)

The PCR conditions for conducting zygosity PCR for rice PE-7 event is given below.

Reagents	Amount to be added
Nuclease-free water	Make up to 25 $\mu$ l
10X reaction buffer (with $MgCl_2$ )	2.5 $\mu$ l
10mM dNTPs	0.5 $\mu$ l
Primer MHTPH-7 (100 ng/ $\mu$ l)	1.0 $\mu$ l
Primer MHIP-5 (100 ng/ $\mu$ l)	1.0 $\mu$ l
Primer MHTPH-8 (100 ng/ $\mu$ l)	1.0 $\mu$ l
Taq DNA polymerase (5 units/ $\mu$ l)	0.5 $\mu$ l
DNA template	2.0 $\mu$ l

Thermal Cycler program:

Temperature	Time	Cycles
95°C	5 minutes	1
94°C	40 seconds	
58°C	40 seconds	35
72°C	1:40minute	
72°C	5 minutes	1
4°C	Hold	---

10 Table 1: Composition of media used in rice transformation

Sr. No.	Medium	Composition	Purpose
1	LB Solid	Tryptone 10 gm/l; Yeast extract 5gm/l; NaCl 10 gm/l; pH 7.0; Agar agar 15gm/l	Bacterial culture
2	LB liquid	Tryptone 10 gm/l; Yeast extract 5gm/l; NaCl 10 gm/l; pH 7.0	Bacteria inoculation.
3	LS liquid	MS major 100 ml/l; MS minor 10 ml/l; MS $CaCl_2$ 10 ml/l; MS Iron 10 ml/l; LS Vitamins Thimine HCL 1mg/l; Myoinositol 100 mg/l; 2,4-D 2.5 mg/l; Maltose 3%; pH 5.8	Bacteria re-suspension
4	LS	MS major 100 ml/l; MS minor 10 ml/l; MS $CaCl_2$ 10 ml/l; MS Iron 10 ml/l; LS Vitamines, Thimine	Seed inoculation

		HCL1mg/l; Myoinositol 100 mg/l; 2,4-D 2.5mg/l; Maltose 3%; pH 5.8; Phytigel 3.0 gm/l	
5	LS-As	MS major 100 ml/l; MS minor 10 ml/l; MS CaCl <sub>2</sub> 10 ml/l; MS Iron 10 ml/l; LS Vitamines, Thimine HCL1mg/l; Myoinositol 100 mg/l; 2,4-D 2.5mg/l; Maltose 3%; pH 5.8; Phytigel 3.0 gm/l; Acetosyringone 100 uM	Co-cultivation
6	LS-H50	MS major 100 ml/l; MS minor 10 ml/l; MS CaCl <sub>2</sub> 10 ml/l; MS Iron 10 ml/l; LS Vitamines, Thimine HCL1mg/l; Myoinositol 100mg/l; 2,4-D 2.5 mg/l; Maltose 3%; pH 5.8; Phytigel 3.0 gm/l; Hygromycin 50 mg/l; Cefotaxime 250 mg/l	Selection.
7	RI- MSB2.5	MS major 100 ml/l; MS minor 10 ml/l; MS CaCl <sub>2</sub> 10 ml/l; MS Iron 10 ml/l; MS Vitamins 10 ml/l; , BAP 2.5 mg/l; , Sucrose 5%; Hygromycin 40 mg/l; Cefotaxime 250 mg/l; pH 5.8; Phytigel 0.6%	Regeneration I
8	RII- MSB2.5	MS major 100 ml/l; MS minor 10 ml/l; MS CaCl <sub>2</sub> 10 ml/l; MS Iron 10 ml/l; MS Vitamins 10 ml/l; , BAP 2.5mg/l; Sucrose 5%; Hygromycin 30 mg/l; Cefotaxime 250 mg/l; pH 5.8; Phytigel 0.3%	Regeneration II and Elongation
9	MSN1.5	MS major 100 ml/l; MS minor 10 ml/l; MS CaCl <sub>2</sub> 10 ml/l; MS Iron 10 ml/l; MS Vitamins 10 ml/l; NAA 1.5mg/l; Sucrose 3%; Hygromycin 20 mg/l; Cefotaxime 250 mg/l; pH 5.8; Phytigel 0.3%	Rooting

SEQ ID NO: 7 consists a part of SEQ ID NO 1 (base 1 to 30), PE-7 T-DNA flanking rice genomic DNA sequence (base 31 to 875), a part of left border (base 876 to 932), e35 S promoter (base 933 to 1559) and a part of *cryIAC* gene coding sequence (base 1560 to 1759).

TATAGGGCTCGAGCGGCCCGGGCAGGTATTATCTAGCAAAAAATAAAGAG  
 ACGCCACGTTATATAAAAAAGGCATCAACAATAAGGTTGGATGTACAATTTATT  
 TTCAAATAGTAATCCTATTGCTAAATCTTCATCCAAAATTGATGAAAATGTACA  
 10 TAATTGTTGTCTCCAACCTGTCAGCATACAACATGAATGAAATCCTTATACTCTTT  
 ACATTTACATTTTTGTAGTTGGCCCAAACTAAATCTAAAATGTTGCCTTGAGTT"  
 CTCAAGCAAGTTTTAAATTTAATAACATGTCTTTGGAAACAAAAGAGCGATACA  
 TCGGCCCTTGTTTCTCATATAAATTGACTTTTACTTCTTTTTTTTTTAACCTTCACA  
 CACACATGCATGCTCTGAAACCAATTACAACACTGCTACCATGTGCCCGTAGCT  
 15 TATGTTCAAGGGGCATGATTTTCATCCACGAGGATCAAAATGCTTGTGGCCACGA  
 ATGTTACCCACATATTTTGTGTCAGTAACTTTTCGATAGAACTCAACAAGGGTAG  
 AGATTTGCCGTTGGTCTCTATCATTATCTCAAATAGGCAAATACCTTCGCATTAC  
 AATCTTTGTAGCCCAAATTTATATGAGAATTGCTAAAGAAAACGACGATTTTCC  
 CCGATCCCCCTCCCAAAAAAAAAAAGAATTGTAACCATCAAATCGTATCGAGAT  
 20 TTGTGCATGAGGGATCTACGCATGTGAAGGGATTAATTGTAACCTATATTATAGC  
 TACAGTATAATTACGCTATAAATATACCGTAGTTATATTTAACTTTTTAAAGA  
 AATTTTGTGACAATTTATTAAGCCTCATTTTAAATTTCCACTATTGAAAACCCAG  
 CATATCACGCTTAGACAACCTTAATAACACATTGCGGGCGTTTTTAATGTACTGA  
 ATTAATTCGAAGCTTGGCTGCAGGTCTGTGAGACTTTTCAACAAAGGGTAATAT  
 25 CCGGAAACCTCCTCGGATTCCATTGCCAGCTATCTGTCACTTTATTGTGAAGAT

AGTGGAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAGG  
 CCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCACCCA  
 CGAGGAGCATCGTGGAAGAAAGACGTTCCAACCACGTCTTCAAAGCAAGTG  
 GATTGATGTGATGGTCCGATTGAGACTTTTCAACAAAGGGTAATATCCGGAAC  
 5 CTCTCGGATTCCATTGCCAGCTATCTGTCACTTTATTGTGAAGATAGTGGA  
 AGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAGGCCATCGTT  
 GAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCACCCACGAGGAG  
 CATCGTGGAAGAAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGATTGAT  
 GTGATATCTCCACTGACGTAAGGGATGACGCACAATCCCACTATCCTTCGCAAG  
 10 ACCCTTCCTCTATATAAGGAAGTTCATTTTCACTTTGGAGAGGACACATGGACAAC  
 AACCCAAACATCAACGAATGCATTCCATACTGCTTGAGTAACCCAGAAGTT  
 GAAGTACTTGGTGGAGAACGCATTGAAACCGGTTACACTCCCATCGACATCTCC  
 TTGTCCTTGACACAGTTTCTGCTCAGCGAGTTCGTGCCAGGTGCTGGGTTCGTTT  
 TCGGACTAGTTGACATCATCTGGGGTAT  
 15

SEQ ID NO: 12 consists of 50 nucleotides from position 851 to position 900 of the nucleotide sequence as set forth in SEQ ID NO: 7

TCCACTATTGAAAACCCAGCATATCACGCTTAGACAACCTTAATAACACAT

SEQ ID NO: 13 consists of 100 nucleotides from position 826 to position 925 of the  
 20 nucleotide sequence as set forth in SEQ ID NO: 7

AATTTATTAAGCCTCATTTTAAATTTCCACTATTGAAAACCCAGCATATCACGCT  
 TAGACAACCTTAATAACACATTGCGGGCGTTTTTAATGTACTGAAT

SEQ ID NO: 14 consists of 150 nucleotides from position 801 to position 950 of the nucleotide sequence as set forth in SEQ ID NO: 7

25 AAACCTTTTAAAGAAATTTTGTGACAATTTATTAAGCCTCATTTTAAATTTCCAC  
 TATTGAAAACCCAGCATATCACGCTTAGACAACCTTAATAACACATTGCGGGCGT  
 TTTTAATGTACTGAATTAATTCGAAGCTTGGCTGCAGGTCT

SEQ ID NO: 15 consists of 1729 nucleotides from position 31 to position 1759 of the nucleotide sequence as set forth in SEQ ID NO: 7

30 ATTATCTAGCAAAAAATAAAGAGACGCCACGTTATATAAAAAAGGCATCAACA  
 ATAAGGTTGGATGTACAATTTATTTTCAAATAGTAATCCTATTGCTAAATCTTCA  
 TCCAAAATTGATGAAAATGTACATAATTGTTGTCTCCAACGTGTCAGCATACAAC  
 ATGAATGAAATCCTTATACTCTTTACATTTACATTTTGTAGTTGGCCAAAAC  
 AAATCTAAAATGTTGCCTTGAGTTCTCAAGCAAGTTTTAAATTTAATAACATGT  
 35 CTTTGGAACAAAAGAGCGATACATCGGCCTTGTTTCTCATATAAATTGACTTT  
 TACTTCTTTTAAAAAACCTTCACACACACATGCATGCTCTGAAACCAATTACA  
 AACTGCTACCATGTGCCCGTAGCTTATGTTCAAGGGGCATGATTTTCATCCACG  
 AGGATCAAAATGCTTGTGGCCACGAATGTTACCCACATATTTTGTGTCAGTAAC  
 TTTGATAGAACTCAACAAGGGTAGAGATTTGCCGTTGGTCTCTATCATTATCTC  
 40 AAATAGGCAAATACCTTCGCATTACAATCTTTGTAGCCCAAATTTATATGAGAA  
 TTGCTAAAGAAAACGACGATTTTCCCCCGATCCCCCTCCCAAAAAAAAAAAGAAT  
 TGTAACCATCAAATCGTATCGAGATTTGTGCATGAGGGATCTACGCATGTGAAG

GGATTAATTGTAACCTATATTATAGCTACAGTATAATTACGCTATAAATATACCG  
 TAGTTATATTTAAACTTTTTAAAGAAATTTTGTGACAATTTATTAAGCCTCATT  
 TAAATTTCCACTATTGAAAACCCAGCATATCACGCTTAGACAACCTTAATAACAC  
 ATTGCGGGCGTTTTTAATGTACTGAATTAATTCGAAGCTTGGCTGCAGGTCTGT  
 5 GAGACTTTTCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCA  
 GCTATCTGTCACTTTATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACAAA  
 TGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGT  
 GGTCCCAAAGATGGACCCCCACCCACGAGGAGCATCGTGGAAAAAGAAGACGT  
 TCCAACCACGTCTTCAAAGCAAGTGGATTGATGTGATGGTCCGATTGAGACTTT  
 10 TCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTG  
 TCACTTTATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCA  
 TTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCA  
 AAGATGGACCCCCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACC  
 ACGTCTTCAAAGCAAGTGGATTGATGTGATATCTCCACTGACGTAAGGGATGAC  
 15 GCACAATCCCACTATCCTTCGCAAGACCCTTCCTCTATATAAGGAAGTTCATTTC  
 ATTTGGAGAGGACACATGGACAACAACCCAAACATCAACGAATGCATTCCATA  
 CAACTGCTTGAGTAACCCAGAAGTTGAAGTACTTGGTGGAGAACGCATTGAAA  
 CCGGTTACACTCCCATCGACATCTCCTTGTCTTGACACAGTTTCTGCTCAGCGA  
 GTTCGTGCCAGGTGCTGGGTTCGTTCTCGGACTAGTTGACATCATCTGGGGTAT  
 20 SEQ ID NO: 16 consists of 939 nucleotides from position 1 to position 939 of the  
 nucleotide sequence as set forth in SEQ ID NO: 7

TATAGGGCTCGAGCGGCCGCCGGGCAGGTATTATCTAGCAAAAAATAAAGAG  
 ACGCCACGTTATATAAAAAAGGCATCAACAATAAGGTTGGATGTACAATTTATT  
 TTCAAATAGTAATCCTATTGCTAAATCTTCATCCAAAATTGATGAAAATGTACA  
 25 TAATTGTTGTCTCCAACTGTCAGCATACAACATGAATGAAATCCTTATACTCTTT  
 ACATTTACATTTTTTGTAGTTGGCCCCAAACTAAATCTAAAATGTTGCCTTGAGTT  
 CTCAAGCAAGTTTTAAATTTAATAACATGTCTTTGGAAACAAAAGAGCGATACA  
 TCGGCCTTGTTTCTCATATAAATTGACTTTTACTTCTTTTTTTTTTAACCTTCACA  
 CACACATGCATGCTCTGAAACCAATTACAACACTGCTACCATGTGCCCGTAGCT  
 30 TATGTTCAAGGGGCATGATTTTCATCCACGAGGATCAAAATGCTTGTGGCCACGA  
 ATGTTACCCACATATTTTGTGTCAGTAACTTTTCGATAGAACTCAACAAGGGTAG  
 AGATTTGCCGTTGGTCTCTATCATTATCTCAAATAGGCAAATACCTTCGCATTAC  
 AATCTTTGTAGCCCAAATTTATATGAGAATTGCTAAAGAAAACGACGATTTTCC  
 CCCGATCCCCCTCCCAAAAAAAAAAAGAATTGTAACCATCAAATCGTATCGAGAT  
 35 TTGTGCATGAGGGATCTACGCATGTGAAGGGATTAATTGTAACCTATATTATAGC  
 TACAGTATAATTACGCTATAAATATAACCGTAGTTATATTTAACTTTTTAAAGA  
 AATTTTGTGACAATTTATTAAGCCTCATTTTAAATTTCCACTATTGAAAACCCAG  
 CATATCACGCTTAGACAACCTTAATAACACATTGCGGGCGTTTTTAATGTACTGA  
 ATTAATTCGAAGCTTG

**What is claimed:**

1. An isolated polynucleotide useful for detection of presence of PE-7 event in a sample comprising rice DNA, wherein the nucleotide sequence of said polynucleotide is selected from the group consisting of SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15 and a complement thereof.  
5
2. A set of synthetic oligonucleotides comprising first and second oligonucleotide useful for detection of presence of PE-7 event in a sample comprising rice DNA, wherein said first oligonucleotide comprises at least 15 nucleotides from position 31 to position 850 of the nucleotide sequence as set forth in SEQ ID NO: 7 and said second oligonucleotide  
10 comprises at least 15 nucleotides from position 900 to position 1759 of the nucleotide sequence as set forth in SEQ ID NO: 7.
3. The set of synthetic oligonucleotides as claimed in claim 2, wherein the first oligonucleotide is as set forth in SEQ ID NO: 8.
4. The set of synthetic oligonucleotides as claimed in claim 2, wherein the second  
15 oligonucleotide is selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 9 and SEQ ID NO: 10.
5. A set of synthetic oligonucleotide useful for detection of presence of PE-7 event in a sample comprising rice DNA, wherein the nucleotide sequence of said oligonucleotide is as set forth in SEQ ID NO: 8 and SEQ ID NO: 4.
- 20 6. A set of synthetic oligonucleotide useful for detection of presence of PE-7 event in a sample comprising rice DNA, wherein the nucleotide sequence of said oligonucleotide is as set forth in SEQ ID NO: 8 and SEQ ID NO: 9.
7. A set of synthetic oligonucleotide useful for detection of presence of PE-7 event in a sample comprising rice DNA, wherein the nucleotide sequence of said oligonucleotide is as  
25 set forth in SEQ ID NO: 8 and SEQ ID NO: 10.
8. A process of detecting the presence of PE-7 event in a sample comprising rice DNA, said method comprising:

a) contacting said sample with a set of synthetic oligonucleotides comprising first and second oligonucleotide useful for detection of presence of PE-7 event in a sample comprising rice DNA, wherein said first oligonucleotide comprises at least 15 nucleotides from position 31 to position 850 of the nucleotide sequence as set forth in SEQ ID NO: 7  
5 and said second oligonucleotide comprises at least 15 nucleotides from position 900 to position 1759 of the nucleotide sequence as set forth in SEQ ID NO: 7.

b) performing amplification reaction; and

c) detecting the presence or absence of an amplicon.

wherein the presence of an amplicon confirms the presence of PE-7 event in said sample.

10 9. The process as claimed in claim 8, wherein the first oligonucleotide is as set forth in SEQ ID NO: 8.

10. The process as claimed in claim 8, wherein the second oligonucleotide is selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 9 and SEQ ID NO: 10.

11. A process of detecting the presence of PE-7 event in a sample comprising rice DNA,  
15 said process comprising:

a) contacting said sample with a polynucleotide probe having nucleotide sequence as set forth in SEQ ID NO: 12, SEQ ID NO: 13 or SEQ ID NO: 14

b) subjecting said sample and said probe to stringent hybridization conditions; and

c) detecting hybridization of said probe to said DNA.

20 wherein the hybridization signals confirms the presence of PE-7 event in said sample.

12. A kit for detection of presence of PE-7 event in a sample, wherein said kit comprising a set of synthetic oligonucleotides comprising first and second oligonucleotide, wherein the nucleotide sequence of said first oligonucleotide is as set forth in SEQ ID NO: 8 and the nucleotide sequence of said second oligonucleotide is selected from the group consisting of  
25 SEQ ID NO: 4, SEQ ID NO: 9 and SEQ ID NO: 10.

13. A kit for detection of presence of PE-7 event in a sample, wherein said kit comprising a polynucleotide probe having nucleotide sequence as set forth in SEQ ID NO: 12, SEQ ID NO: 13 or SEQ ID NO: 14
- 5 14. A transgenic plant produced according to the method of claim 3, wherein said plant comprises the polynucleotide sequence as set forth in SEQ ID NO: 7
15. A plant cell or a progeny thereof, wherein the plant cell, or the progeny thereof comprises the polynucleotide sequence as set forth in SEQ ID NO: 7

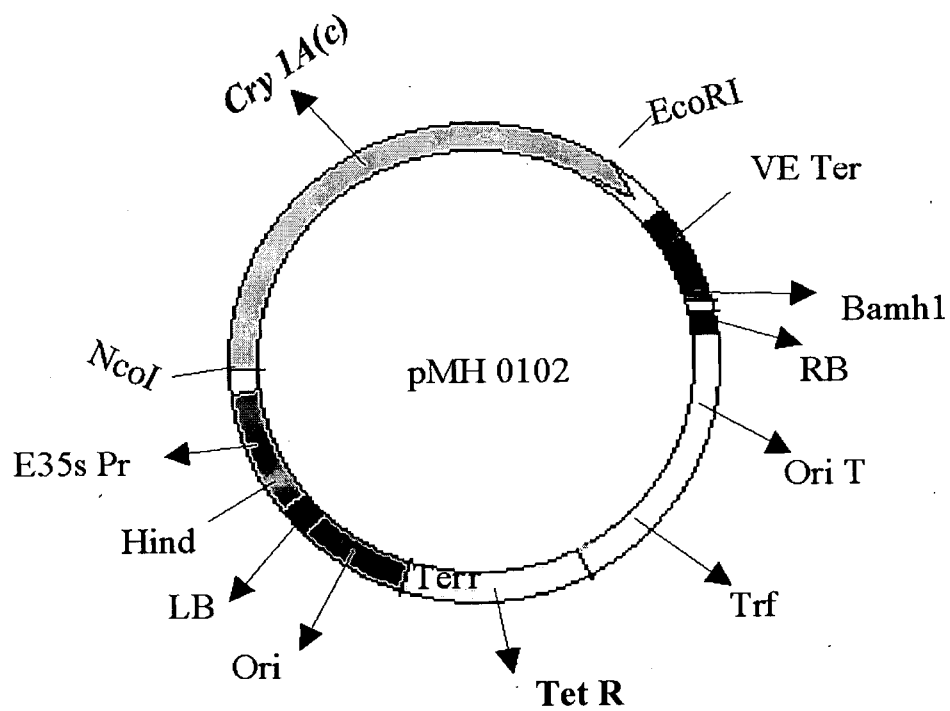


Figure 1

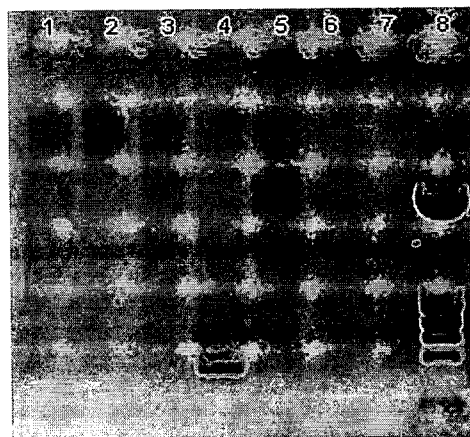
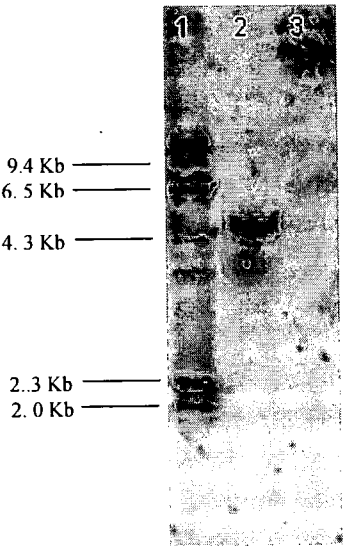


Figure 2

A



B

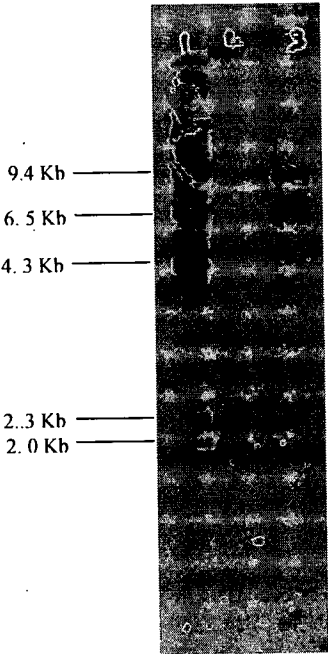


Figure 3